

AMENDMENTS TO THE CLAIMS

1-28. (cancelled)

29. (withdrawn) An assay to identify molecular markers linked to phenotypic stability of a chondrocyte cell population comprising:

- a) providing a suspension of isolated or expanded cells and determining positive and negative markers thereof,
- b) injecting intramuscularly or subcutaneously in a non-human animal said suspension of isolated or expanded cells in an iso-osmotic liquid, the same suspension comprising articular chondrocytes in an amount equivalent to at least 1×10^6 chondrocytes as applied to immune-deficient mice,
- c) allowing the formation of cartilaginous tissue,
- d) sacrificing the animal,
- e) evaluating the in vivo formed cartilage histologically for stable, non-vascularised cartilage, and
- f) identifying positive or negative molecular markers of those isolated or expanded cells which formed stable, non-vascularised cartilage in vivo, as evaluated in step e).

30. (withdrawn) An assay to identify molecular markers according to claim 29, comprising using freshly isolated or serially passaged cells and using differential gene expression analysis methods selected from the group consisting of differential

display, subtractive hybridization, subtracted libraries or cDNA chips and cDNA arrays to identify said positive or negative molecular markers of those isolated or expanded cells which formed stable, non-vascularized cartilage in vivo.

31. (currently amended) A method to identify cells having chondrocyte phenotypic stability comprising:

(1) determining the expression by said cells of a positive marker of chondrocyte phenotypic stability which is BMP-2 and/or FGFR-3 and/or a marker co-detectable with these markers identifiable by a method comprising the steps of:

- a) providing a suspension of isolated or expanded cells and determining the expression of a positive marker thereof,
- b) injecting intramuscularly or subcutaneously in a non-human animal said suspension of isolated or expanded cells in an iso-osmotic liquid, the same suspension comprising articular chondrocytes in an amount equivalent to at least 1×10^6 chondrocytes as applied to immune-deficient mice,
- c) allowing the formation of cartilaginous tissue,
- d) sacrificing the animal,
- e) histologically evaluating the in vivo formation of stable, non-vascularised cartilage, and
- f) identifying a marker which is co-expressed with ~~whether said expression of said~~

positive markers ~~is-linked to the to-the~~ formation of stable, non-vascularised cartilage in vivo ~~by said isolated or expanded cells~~, as evaluated in step e); and
(2) identifying said cells expressing said BMP-2 and/or FGFR-3 and/or said marker co-detectable with these markers as cells having chondrocyte phenotypic stability.

32. (currently amended) A method to identify cells having chondrocyte phenotypic stability according to claim 31 further comprising:

1)-determining that activin-like kinase-1 (ALK-1) is not expressed by said cells, and/or a marker is not expressed by said cells which is a marker co-detectable with said ALK-1 marker identified by a method comprising the steps of:

- a) providing a suspension of isolated or expanded cells and determining a negative marker thereof,
- b) injecting intramuscularly or subcutaneously in a non-human animal said suspension of isolated or expanded cells in an iso-osmotic liquid, the same suspension comprising articular chondrocytes in an amount equivalent to at least 1×10^6 chondrocytes as applied to immune-deficient mice,
- c) allowing ~~the~~ formation of cartilaginous tissue,
- d) sacrificing the animal,
- e) evaluating ~~the~~ in vivo formed cartilage histologically for stable, non-vascularised cartilage, and

f) identifying a marker which is co-expressed with ~~whether the expression of said~~
negative molecular marker ~~is linked to the formation of stable, non-vascularised~~
cartilage by said isolated or expanded cells in vivo, as evaluated in step e); and
~~and/or specific reporter constructs comprising a promoter of said markers.~~

2) identifying said cells not expressing said ALK-1 marker and/or said marker co-
detectable with said negative marker, as a cell population having chondrocyte phenotypic
stability

33. (currently amended) The method to identify cells ~~with~~having chondrocyte phenotypic
stability according to claim 31, comprising

- a) hybridising to messenger RNA from cells of said cells, sets of DNA probes
provided on DNA arrays or DNA chips, said DNA probes being probes of said
positive markers for chondrocyte phenotypic stability BMP-2 and/or said FGFR-3
and/or said markers co-detectable with these markers.
- b) ~~Identifying~~ identifying those cells which hybridise with said probes of said
positive markers for chondrocyte phenotypic stability.

34. (currently amended) The method to identify phenotypically stable chondrocytes
according to claim 31, said method comprising detecting said ~~set of positive markers~~
marker in ~~a~~ cells from a cartilage biopsy, after at least one passage.

35. (currently amended) The method to identify phenotypically stable chondrocytes according to claim 31, which method is used to monitor passage by passage cell expansion and/or to predict when cell expansion must be stopped and/or to recover cells that have already lost their phenotypic stability only when needed and/or to provide a means for quality control of cells to be used for autologous cell transplantation and/or selecting from a cell population only those cells that retain their chondrocyte phenotypic stability.
36. (currently amended) The Method according to claim 35, comprising sorting said phenotypically stable chondrocytes via monoclonal or polyclonal antibodies against said positive markers or said co-detectable marker ~~co-detectable markers~~.
37. (cancelled)
38. (cancelled)
39. (withdrawn) Cells identified according to a method comprising:
- a) hybridising to messenger RNA from cells, sets of DNA probes provided on DNA arrays or DNA chips, said DNA probes being probes of positive and negative markers for chondrocyte phenotypic stability,

- b) Identifying those cells which hybridise with said positive markers and do not hybridise with said negative markers for chondrocyte phenotypic stability.

40. (withdrawn) Cells selected according to a method comprising detecting the expression of molecular markers of chondrocyte phenotypic stability selected from the group of:

- BMP-2 and/or FGFR-3 and/or markers co-detectable with these markers identifiable by the method of claim 29 and/or specific reporter constructs associated with these markers, which markers are positive markers for chondrocyte phenotypic stability.
- expressed activin-like kinase-1 (ALK-1) as a marker negatively associated with chondrocyte phenotypic stability, and/or markers co-detectable with these markers identifiable by the method of claim 29 and/or specific reporter constructs comprising a promoter of said markers;

wherein those cells are selected in which expression of positive markers and absence of markers negatively associated with phenotypic stability is detected.

41. (withdrawn) An assay to predict the outcome of autologous cell transplantation comprising

- a) providing isolated or expanded chondrocytes

- b) subcutaneously or intramuscularly injecting in a non-human animal of a suspension of said cells in an iso-osmotic liquid, said suspension comprising articular chondrocytes in an amount equivalent to at least 1×10^6 chondrocytes as applied to immune-deficient mice.
- c) allowing the formation of cartilaginous tissue,
- d) sacrificing the animal,
- e) evaluating the in vivo formed cartilage histologically for stable, non-vascularised cartilage;

whereby the formation of stable, non-vascularised cartilage is indicative of a positive outcome of autologous cell transplantation using said isolated or expanded chondrocytes.

42. (cancelled)

43. (previously presented) A method of transplanting cells to a connective tissue site in a patient or a method of seeding with cells any prosthetic device intended to be anchored into a mammal, wherein said cells are cells which retain their chondrocyte phenotypic stability and which are identified according to the method of any one of claims 31 to 33.

44. (previously presented) A method of transplanting cells to a connective tissue site in a

patient or a method of seeding with cells any prosthetic device intended to be anchored into a mammal wherein said cells are cells which retain their chondrocyte phenotypic stability and which are selected according to the method of claim 35.

45. (previously presented) A therapeutic composition for humans including cells identified according to claim any one of claims 31 to 33, optionally further including at least a pharmaceutically acceptable carrier and/or a growth factor.
46. (withdrawn) A therapeutic composition for humans including cells selected according to claim 35, optionally further including at least a pharmaceutically acceptable carrier and/or a growth factor.
47. (withdrawn) A diagnostic comprising DNA chips comprising DNA probes of positive and negative markers for chondrocyte phenotypic stability identified according to the method of claim 29.
48. (cancelled)
49. (withdrawn) A cell culture exhibiting chondrocyte phenotypic stability in which the cells express a ratio of BMP-2 and/or FGFR-3 as molecular markers positively

associated with chondrocyte phenotypic stability and/or markers co-detectable with these markers identifiable by the method of claim 29 and/or specific reporter constructs associated with these markers to activin-like kinase-1 (ALK-1) as a molecular marker negatively associated with chondrocyte phenotypic stability and/or markers co-detectable with this marker identifiable by the method of claim 29 and/or specific reporter constructs comprising a promoter of said negative marker, which is greater than 1.

50. (withdrawn) A cell culture exhibiting chondrocyte phenotypic stability which culture does not express activin-like kinase-1 (ALK-1) and/or markers co-detectable with this marker identifiable by the method of claim 29 and/or specific reporter constructs comprising a promoter of said markers.

51. (previously presented) The method of claim 33, wherein said DNA probes are provided on a DNA array or a DNA chip.

52. (withdrawn) The method of claim 39, wherein said DNA probes are provided on a DNA array or a DNA chip.

53. (withdrawn) The diagnostic of claim 47, wherein said positive or negative markers are

DNA probes.

54. (withdrawn) The cell culture exhibiting chondrocyte phenotypic stability, according to claim 49, wherein said ratio is greater than 2.

55. (currently amended) The method to identify cells ~~with~~ having chondrocyte phenotypic stability according to claim 32, comprising

a) hybridising to messenger RNA from cells, sets of DNA probes provided on DNA arrays or DNA chips, said DNA probes being probes of said negative marker for chondrocyte phenotypic stability ALK-1 and/or said marker co-detectable with ALK-1.

b) ~~Identifying~~ identifying those cells which hybridise with said probe of said positive marker for chondrocyte phenotypic stability.

56. (currently amended) The method to identify cells having chondrocyte phenotypic stability of claim 31, said method comprising determining the expression of a positive marker of chondrocyte phenotypic stability which is BMP-2.

57. (currently amended) The method to identify cells having chondrocyte phenotypic stability of claim 31, said method comprising determining the expression of a positive

marker of chondrocyte phenotypic stability which is FGFR3.

58. (previously presented) The method to identify cells having chondrocyte phenotypic stability of claim 33, wherein said positive marker of chondrocyte phenotypic stability is BMP-2.

59. (previously presented) The method to identify cells having chondrocyte phenotypic stability of claim 33, wherein said positive marker of chondrocyte phenotypic stability is FGFR3.

60. (new) The method to identify cells having chondrocyte phenotypic stability according to claim 31, further comprising further comprising determining that activin-like kinase-1 (ALK-1) is not expressed by said cells.